PRECLINICAL DATA SUPPORTING THE INITIATION OF THE EDIT-301 PHASE I/II RUBY CLINICAL TRIAL FOR THE POTENTIAL TREATMENT OF SICKLE CELL DISEASE

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EHA2021
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Naturally occurring HbF-inducing mutations support clinical relevance and safety of editing

αα ββ ββ ββ
HbS HbF

Sickle hemoglobin (HbS)

CRISPR-Cas12a editing at the HBG1 and HBG2 promoter regions induces HbF expression

Unedited CD34+ cells\textsuperscript{a} from patients with SCD

EDIT-301: Edited CD34+ cells\textsuperscript{a} from patients with SCD

HbF production reduces RBC sickling by inhibiting formation of HbS fibers at low oxygen levels

\textsuperscript{a}CD34\textsuperscript{+} hematopoietic stem and progenitor cell

HS: hypersensitive site; LCR: locus control region; TSS: transcriptional start site

Adapted from Higgs, Engel and Stamatoyannopoulos. Lancet 2012

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The page contains information about EDIT-301 Editing, specifically focusing on highly specific and potent CRISPR-Cas12a enzyme. The content is divided into three sections:

1. **Editing Specificity**
   - Gotta et al. Cold Spring Harbor 2019
   - AsCas12a is Highly Specific primarily due to its DNA target engagement mechanism that is distinct from SpCas9.

2. **Editing Efficiency**
   - De Dreuzy et al, ASH 2019
   - Editas engineered AsCas12a RNP demonstrates high editing efficiency in CD34+ cells.

3. **Indel Profile**
   - De Dreuzy et al, ASH 2019
   - De Dreuzy et al, ASGCT 2018
   - AsCas12a staggered cut generate larger deletions than SpCas9, leading to higher HbF induction at the HBG locus.

The text also includes a note about matching target sites and the compatibility of PAM's for different nucleases (20-Na).
High level of editing and robust HbF induction in edited CD34+ cells from normal donors and patients with Sickle Cell Disease (SCD)

Efficient editing

Robust ex vivo HbF expression

Unedited cells did not undergo electroporation
EDIT-301-derived RBCs have reduced sickling and improved rheological properties versus unedited SCD-derived RBCs

**Reduced sickling**

- Unedited SCD-derived RBCs
- EDIT-301 (edited SCD) derived RBCs

**Improved rheological behavior**

- Normal donor-derived RBCs
- Unedited SCD-derived RBCs
- EDIT-301 (edited SCD)-derived RBCs

- Oxygen level: 4%
- $R^2=0.9321$

**Mean HbF (%):**
- Unedited: 19.9
- EDIT-301: 53.8

*When placed in microfluidic channels, mimicking blood flow in microvasculature, at a range of oxygen levels*

*When exposed to sodium metabisulfite*
Consistent and robust large-scale manufacturing of edited CD34+ cells from normal donors

Robust large-scale manufacturing

- Recovery (n=8)
- Viability (n=8)
- CD34 Purity (n=8)
- Editing (n=8)

Consistent editing profile

- Indel Length
  - Large scale Edited batch (n=5)

Deletions are represented as negative values; Insertions as positive values.
Cas12a RNP is highly specific and no off-target editing was detected in large scale manufacturing batches.

Discovery Phase

- **In silico modeling**
  Predicted sites base on sequence

- **Digeneome-Seq**
  Cleaved sites on naked DNA

- **GUIDE-Seq**
  Cleaved sites in cellular context

Verification Phase

- **Targeted Sequencing in CD34+ cells**
  Editing activity at “discovered” candidate sites

No off-target editing observed in Cas12a treated CD34+ cells.
No detectable unintended globin transcript variants in edited CD34-derived erythroid precursors (Large scale batches)

Direct RNA-seq analysis conducted using the Oxford Nanopore System on erythroid cells RNA harvested after 7 days of erythroid differentiation

Large-scale manufacturing –
Unedited CD34 derived Erythroid Precursor

Unedited Erythroid Precursors
Edited Erythroid Precursors

N=3 CD34 normal donors
No detectable unintended globin protein variants in edited CD34-derived RBCs (Large scale batches)

Large-scale manufacturing –
Unedited CD34 derived erythroid cells

Large-scale manufacturing –
Edited CD34 derived erythroid cells

N=3 CD34 normal donors

UHPLC-MS analysis of erythroid cells lysates harvested after 18 days of erythroid differentiation
Infusion of edited CD34+ cells manufactured on a large scale to NSG mice leads to polyclonal engraftment with no lineage skewing

Efficient editing maintained in vivo

No lineage skewing after engraftment

Stable polyclonal engraftment

Bone marrow at 20 weeks post-infusion (Female NSG mice)

Representative data from one NSG mouse. Each color or color shade represents an individual indel signature.

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Conclusions

High levels of editing were achieved in CD34+ cells using highly specific Cas12a enzyme, leading to **potentially therapeutically relevant levels of HbF** expression.

EDIT-301 (edited SCD)-derived RBCs demonstrated a **significant reduction in sickling** and **improved rheological properties**.

**Large-scale process suitable for use in clinical manufacturing** led to consistent editing without off-target and unintended HBG variants. Infusion of the edited cells in mice gave rise to multilineage and polyclonal engraftment with persistence of high levels of editing.

These results support the **initiation of the RUBY clinical trial**, a phase 1/2 study of EDIT-301 to treat patients with severe SCD (NCT#04853576).
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